Original Article

Investigating Effects of Curcuma Longa on the Liver Enzymes and Histopathological Examination in Chemical Induced Liver Injury in Rats

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ABSTRACT

Objective: The present research investigated the hepatoprotective and histoprotective potential of Curcuma longa (CL) against chemical induced liver injury in laboratory male Wistar rats.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Animal House, Al-Tibri Medical College and Sindh Agriculture University from September 2016 to January 2017.

Materials and Methods: 60 adult male rats (Wistar strain), of 150- 250 grams, were randomly divided into 3 groups. Group A. Control (n=20) Rats were given isotonic saline (0.9%) orally daily for 4 weeks, Group B. (n=20) Rats were given Carbon tetrachloride (CCl_4) orally on alternate day for 4 weeks, Group C. (n=20) Rats were fed received Carbon tetrachloride (CCl_4) + Curcuma longa (250 mg/kg) orally on alternate days for 4 weeks. Blood samples were collected by cardiac puncture. Liver tissue 3-5µ thick sections were stained with H & E stain and examined by light microscopy. Statistical analysis was performed on Statistix 9.0 (USA) (P-value of ≤ 0.5)

Results: The present study shows hepatoprotective and histoprotective potential of Curcuma longa (CL) against chemical (carbon tetrachloride) induced liver injury. Curcuma longa improved Liver enzymes, serum superoxide dismutase, glutathione peroxidase, catalase and liver histology (P <0.05).

Conclusion: Curcuma longa exerts hepatoprotective and histoprotective effects, and mitigates oxidative damage induced by carbon tetrachloride.

Key Words: Curcuma longa, Carbon tetrachloride, Liver enzymes, Histology

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INTRODUCTION

Curcuma longa (CL) is an herbal rhizome. It is a perennial herb. Botanically, it belongs to a native South Asian family called the "Zingiberaceae".

Publicly it is known as the turmeric.[1] Turmeric is used in food cooking due to its culinary odor and taste. Turmeric is also used as "herbal remedy" because its medicinal properties are known to public since centuries back. In Folk medicine, the CL is used as digestive and carminative, stomachic and tonic. It is also used for the disease such as the worm infestations, urinary disorders, asthma, and gonorrhea.[2] Curcuma longa is reported of having anti-tumor potential,[3] anti bacterial and antimicrobial activity,[4] anti-inflammatory[5], anti-oxidant[6] and wound healing[7] properties. Its gastro protective effects have also been reported previously.[8] Similarly, in traditional medicine, several herbs had been used in experimental research for induced diseases such as chronic liver disorders, liver cirrhosis, and chemical induced liver injuries.[9,10] Carbon tetrachloride (CCl_4) is chemical agent which has been used in experimental animal studies to evaluate various herbs of their medicinal potential, so that they may be used for the human purpose. The CCl_4 is a hepatotoxic agent. It has been used in laboratory animals to induce liver injury.[11] Mechanism of CCl_4 cell injury is not well understood. One postulated mechanism is the formation of free oxygen radicals

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called reactive oxygen species (ROS). Free reactive oxygen radicals react with the cell membrane and induce lipid peroxidation and consequent upon the cell membrane destruction.[11, 12] Liver tissue architecture disruption results in loss of physiological homeostasis of hepatocyte.[13] As the cell membrane is injured, the cytoplasmic and mitochondrial enzymes are released into the blood capillaries.[14] Liver enzymes are clinical biomarkers of liver injury and their blood levels correlate with the extent of tissue injury. Liver enzymes also indicate cytoplasmic and mitochondria injury at sub cellular levels. Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) are enzymes of cytoplasmic compartment. While the lactate dehydrogenase (LDH) indicates mitochondrial membrane injury.[11,15] Some of previous studies[7,8] have been conducted with CL against the CCl4 induced liver injury. The present experimental study investigated the hepatoprotective and histoprotective potential of Curcuma longa against Carbon tetrachloride induced liver injury in a male Wistar rat model. The present study hypothesized that the Curcuma longa has no effect against the Carbon tetrachloride induced liver injury.

MATERIALS AND METHODS

The present experimental research study was conducted at the Animal house of Al-Tibri Medical College and Sindh Agriculture University from September 2016 to January 2017. Rats were selected through non probability (purposive) according to selection criteria of inclusion and exclusion. Adult male rats (Wistar strain) of 150- 250 grams were the inclusion criteria. Female rats, different weight, not eating well and feeling sick were excluded. Our animal house is well equipped. Animals were kept in steel cages at an optimal temperature (22- 25 ^{0}C), humidity 55-60% and 12/12 hours dark and light cycle. Animals were observed on daily basis. 24 hours access to chow diet and clean pure water was strictly followed. 60 male rats were randomly divided into 3 groups.

Group A. Control (n=20) Rats were given isotonic saline (0.9%) orally daily for 4 weeks,

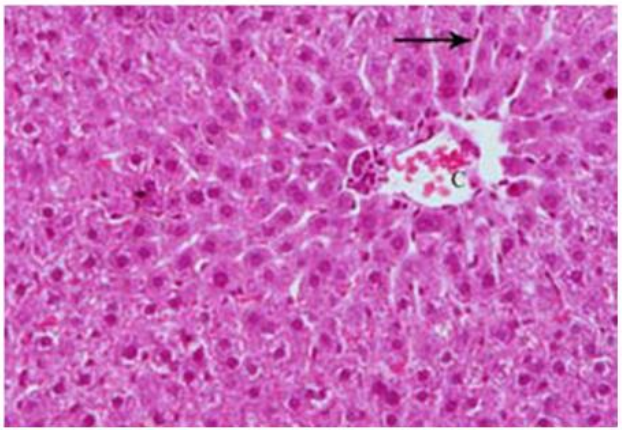
Group B. (n=20) Rats were given Carbon tetrachloride (CCl_4) orally on alternate day for 4 weeks;

Group C. (n=20) Rats were fed received Carbon tetrachloride (CCl_4) + Curcuma longa (250 mg/kg) orally on alternate days for 4 weeks.

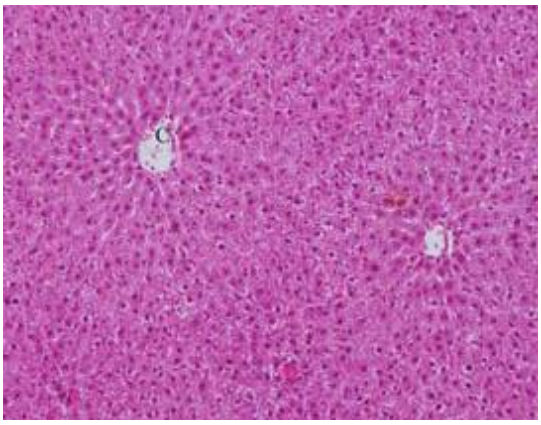
This protocol was strictly followed. CCl_4 was ordered before conducting study. The World Scientific store provided the CCl_4. Olive oil was used as vehicle. The CCl_4 was mixed in olive oil in 1:1 ratio. This was given orally 1.9 ml/kg orally on alternate days for consecutive 4 weeks.[15] Curcuma longa was purchased and administered at dose of 250 mg/kg orally on alternate days for 4 weeks.[16] At the end of experiment period, blood samples were collected by cardiac puncture (24 hours after experiment). Blood samples were centrifuged at 4000 rpm for 10 minutes. Sera were separated out in tubes for biochemical investigation. Biochemical analysis of liver enzymes; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and Gamma glutamyl transferase (ϒ-GT) was performed on Hitachi Roche Chemistry Analyzer. The rats were sacrificed as described previously.[17] Laparotomy was performed by a trained veterinary technician. Liver was retrieved and freed from peritoneum and shifted to container containing the formaldehyde. Tissue pieces were embedded in paraffin. 3-5µ thick tissue sections were cut by microtome and stained with H & E stain. Histological slides were examined by light microscopy. Statistical analysis was performed on Statistix 9.0 (USA). Continuous variables were analyzed by one way ANOVA and descriptive statistics. Tukey Cramer post Hoc testing was used for analysis of difference between groups. Results were presented as mean ± SD. Statistical significance was defined as P-value of ≤ 0.5.

RESULTS

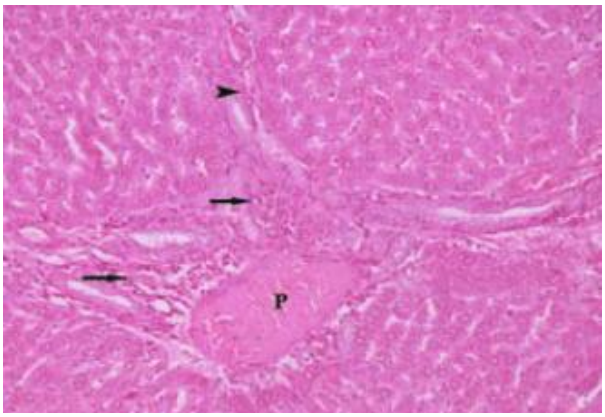
The present experimental rat model research shows beneficial effects of Curcuma longa (CL) against chemical induced liver injury. The mitochondrial and cytoplasmic enzymes of liver shows low rise in CL treated rats. ALT, AST, ALP and LDH were raised significantly in the group B- CCl_4 treated rats. The same parameters show a decline which was given CL along with CCl_4 in group C compared to controls group A ($P<0.05$). Curcuma longa reveals hepatoprotective effects against the CCl_4 induced liver injury (Table 1). Serum Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) levels reveal a significantly rising pattern in CL treated rats. The rise in enzyme antioxidants is a new finding which shows the CL helps in annihilating the free radicals ($P<0.05$, Table 1). Histopathological examinations show histoprotective effects of the CL. Photomicrograph 1 and 2 shows the normal liver tissue showing hepatocytes arranged in cords with portal triad. Central venule is also visible.



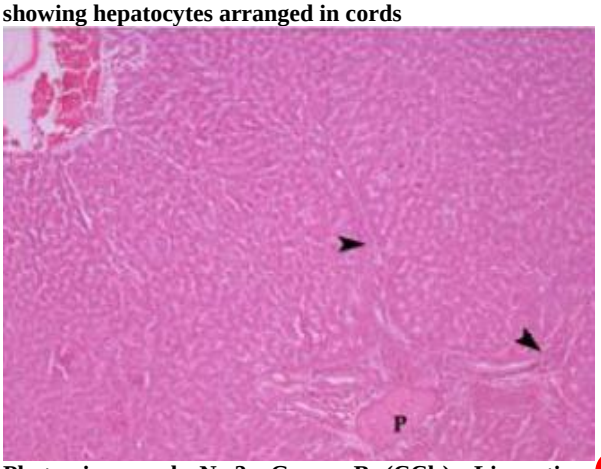
Photomicrograph No.1: Control- Normal liver tissue showing hepatocytes arranged in cords



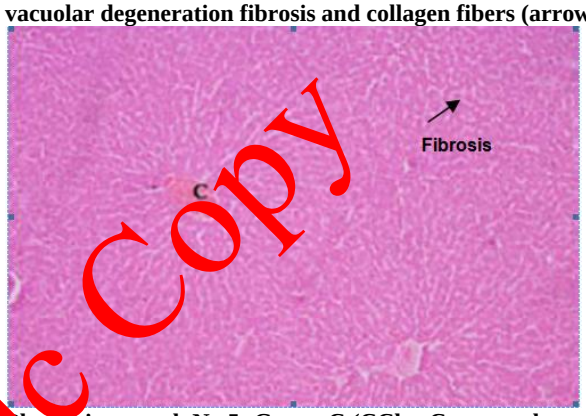
Photomicrograph No.2: Control- Normal liver tissue showing hepatocytes arranged in cords



Photomicrograph No.4: Group B (CCl_4)- Liver tissue section showing inflammatory infiltrates, necrosis, vacuolar degeneration fibrosis and collagen fibers (arrow)



Photomicrograph No.3: Group B (CCl_4)- Liver tissue section showing dilation and congestion of portal and central vein. Arrow head indicate areas of inflammation, necrosis, vacuolar degeneration and fibrosis.

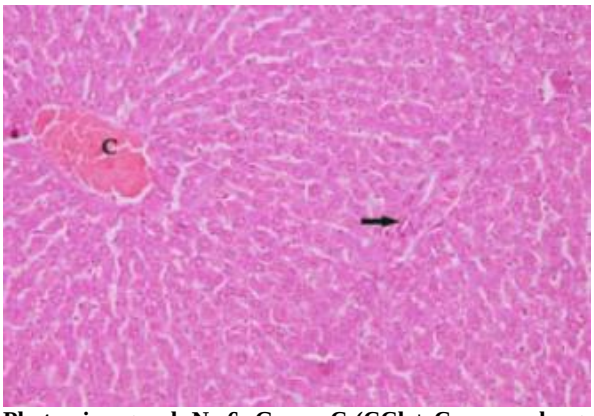


Photomicrograph No.5: Group C (CCl_4+ Curcuma longa)- Liver tissue section showing the hepatoprotective effects of Curcuma longa. Central venule (c) is normal. Fibrosis is minimized at the corners of liver specimen.

Table No.1: Liver enzymes and anti oxidant enzymes in controls and experimental rats (n=60)

	Group A (Controls)	Group B (CCl_4)	Group C (CCl_4 + Curcuma)	P-value
Alanine transaminase (U/L)	37.8±8.8	69.5±13.2	63.6±11.0	0.0001
Aspartate transaminase (U/L)	26.7±4.1	51.1±.5	39.5±11.5	0.009
Alkaline phosphatase (U/L)	73.8±13.0	143.5±29.5	115.5±37.5	0.029
Lactate dehydrogenase (U/L)	110.5±17.3	170.5±25.3	153.5±31.1	0.002
ϒ-Glutamyl transferase (U/L)	34.6±5.12	77.8±6.0	56.5±21.8	0.021
Bilirubin (mg/dl)	0.5±0.15	1.51±0.31	1.43±0.12	0.041
Creatinine (mg/dl)	0.7±0.11	1.5±0.13	1.2±0.31	0.001
Superoxide dismutase (U/ml)	133.51±32.56	76.35±13.94	131.81±13.15	0.035
Glutathione peroxidase (nM/min/mL)	134.30±33.83	88.03±23.13	123.5±9.08	0.0001
Serum Catalase (nM/min/mL)	407.54±81.32	172.71±92.33	267.35±32.05	0.0001

The Liver sections of the control group show intact central venules and hepatocytes cords. Destruction of tissue architecture by CCl4 is seen in the Photomicrograph 3 and 4. Liver tissue section showing of dilation and congestion of portal and central vein is seen. Arrow head indicate areas of inflammation, necrosis, vacuolar degeneration collagen, and fibrosis. Congestion of central venule, sinusoids and portal triad were observed. Centrilobular hepatocytes revealed hydropic changes and necrosis. The midzonal and peripheral hepatocytes revealed vacuolar degeneration, necrosis and fatty changes in CCl_4 treated liver tissue. Diacerein treated groups 5 and 6 exhibited amelioration of tissue architecture. Diacerein treated showed normalization of tissue. Liver tissue section showing the hepatoprotective effects of Curcuma longa is seen. Central venule (c) is normal. Fibrosis is minimized at the corners of liver specimen.



Photomicrograph No.6: Group C (CCl_4+ Curcuma longa) Liver tissue section shows the hepatoprotective effects of Curcuma longa. Central venule (c) is normal. Fibrosis is minimized at the corners of liver specimen.

DISCUSSION

The present experimental study analyzed the Curcuma longa (CL) of its ameliorating effects of biochemical and histopathological parameters in carbon tetrachloride (CCl_4) induced liver injury. CL is a commonly used food additive. CL is a rhizome and its active constituent is known as the Curcumin. The Curcumin exerts anti oxidant effects. It enhances the apoptosis of injured hepatocytes. This mechanism has been proposed as down regulating the inflammation, hepatocyte injury and fibrogenesis.[18] The CCl_4 treated rat's revealed severe liver injury as indicated by a rise in liver enzymes and histopathological examination. The findings of CCl_4 induced liver injury are in keeping with previous study.[19] This previous study[20] rise in liver enzymes and destroyed tissue architecture. Presence of elevated cytoplasmic (ALT, AST, ALP) and mitochondrial (LDH) enzymes of liver indicate hepatocellular injury, as a result of hepatocyte membrane injury.[20] The present study used ethanol extract of Curcuma longa administered orally at dose of 250 mg/kg body weight. Active ingredients of CL by a previous study[21] were reported as Curcumin (flavonoids) and volatile oils such as the atlantone, zingiberene and tumerone. A previous study[22] reported the CL extracts exert direct free radical scavenging activity, enhances glutathione levels, and augments glutathione peroxides activity thereby accelerating the detoxification. In present study, the serum Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) levels reveal a significantly rising pattern in CL treated rats. The rise in enzyme antioxidants is a new finding which shows the CL helps in annihilating the free radicals ($P<0.05$, Table 1). These findings are consistent with previous studies.[7,8] The findings are also in keeping with recent study.[21] Volatile oils of CL exerts anti inflammatory activity.[23] Above findings of tissue protection by CL are consistent with the present study. Elevated cytoplasmic (ALT, AST, ALP) and mitochondrial (LDH) enzymes of liver in CCl_4 treated rats indicate the hepatocellular injury.[24,25] Histopathological findings of CCl_4 liver specimens correlate with the rise in liver enzyme. The findings are in keeping with recent study.[26] Histopathological examinations show histoprotective effects of the CL. Destruction of tissue architecture by CCl4 (Photomicrograph 3 and 4) revealed dilation and congestion of portal and central vein, inflammation, necrosis, vacuolar degeneration collagen, and fibrosis. Congestion of central venule, sinusoids and portal triad were observed. Centrilobular hepatocytes revealed hydropic changes and necrosis. Diacerein normalizes these histopathological changes. In Diacerein treated liver rats, the tissue architecture was normalized and Fibrosis was minimized at the corners of liver specimen (Photomicrograph 5 and 6). Our findings are in agreement with previous studies.[27,28] Our findings are also in agreement with another previous study.[21] They conducted study to analyze the hepatoprotective effects of CL in thioacetamide induced liver injury and cirrhosis. The findings are also in keeping with recent study by Singh et al 2017.[26] Statistical analysis shows the CL treated rats revealed significant difference when compared with CCl4 treated rats; hence the null hypothesis was rejected. The hepatoprotective and histoprotective effects of CL were accepted. Thus the present study concluded that the CL may be used in drug and chemical induced liver injury, however, further research is needed.

CONCLUSION

The Curcuma longa exerts hepatoprotective and histoprotective effects, and mitigates oxidative damage induced by carbon tetrachloride. The present study concludes the Curcuma longa may be used in drug and chemical induced liver injury; however, further research studies are warranted.

Author's Contribution:

Concept & Design of Study: Aftab Ahmed Shaikh
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Data Analysis: Shomail Saeed Siddiqui
Kashif Rasheed Shaikh
Revisiting Critically: Mumtaz Ali Qureshi
Munawar Ali Kalhoro
Final Approval of version: Aftab Ahmed Shaikh

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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